A New Method of Studying the Chemistry of Rancidity*

By LOWELL B. KILGORE

THE Mayonnaise Manufacturers' Association established a cooperative research fellowship at the Bureau of Chemistry and Soils in Washington, D. C., August 1, 1930. This fellowship was designed to prosecute certain investigations concerning means of increasing the stability of commercial mayonnaise. Such a program must, of course, include many subdivisions, such as studies made upon methods of analyses, fundamentals of oil-in-water emulsions, causes of the spoilage of the constituents and other closely related subjects. The particular division of this program of investigation that is of mutual interest to us is that of the various causes of the spoilage of the vegetable oils used in the manufacture of mayonnaise.

Although the spoilage of mayonnaise oils has been placed in the category as a subdivision it is nevertheless a very important part of the program. The causes of the spoilage of mayonnaise in general may be divided into two main headings:

(1) The deterioration of the emulsifying agent, and (2) the development of rancidity in the oil phase.

It is recognized that these causes are not entirely independent of each other but that the total spoilage is a cumulation of the two classes of reactions. However, it is believed that if an emulsifying agent could be found which did not change in its emulsifying power or allow the film to break and if an oil could be used which did not develop objectionable odors and flavors, the problems of distributing and marketing mayonnaise would have largely been solved. For this reason the above classification into the two groups of problems has been made as a working basis for the fundamental part of our research program.[1]

Mayonnaise is a fortunate emulsion when considered from the viewpoint of the tendency of its constituents toward spoilage. Consider the egg and the oil constituents. If they are placed in separate containers on the same shelf and for the same length of time as a sample of mayonnaise made from the same lot of eggs and oil, the mayonnaise will be edible long after the eggs and oil have spoiled. The bulk of the constituents of mayonnaise may therefore be said to be perishable, yet mayonnaise is sold as a fairly stable foodstuff.

It would justly be considered presumptuous to come before this group of well seasoned oil chemists whose organization is well established, to attempt to add to their store-house of information. In the short time the Mayonnaise Fellowship plan has been in operation, effort has been made to cover the literature on the subject of rancidity and to become familiar with the enormous amount of work which has been done in this field of investigation. It does not seem possible that anyone could do this without realizing the size of the problem at hand in connection with the subject of prevention of rancidity in fats and oils. However, some pioneering experiments have been made in an attempt to find a method of removing the reaction products produced during the rancidification of an oil or fat. It was thought that if a definite chemical reaction could be found which would permit solid derivatives to be formed with those compounds in the oils which give rise to the offending flavors and odors, and thus remove them, a valuable means of studying the actual chemis try of rancidification would be at hand. The existence of aldehydes in rancid fats has been suggested by numerous workers in the field. However, the proof of the existence of aldehydes as such in the rancid oils has largely been indirect.

*Presented before American Oil Chemists' Society.

[1] *Kilgore, "Report on Mayonnaise Fellowship," The Canner, 72, 30, March 14, 1931.*

Scala[2] identified several organic acids in fats by precipitating them as barium salts. Nicolet[3] and others have also identified various acids in rancid fats. The actual presence of the corresponding aldehydes has yet to be proved. Furthermore, the possibility of the existence of other carbonyl compounds which do not readily oxidize to fatty acids cannot be investigated by the above methods. It seems highly desirable, therefore, that some method of removing such compounds by forming derivatives in some manner, be developed.

A careful study has been made of the various reagents used for the formation of derivatives of aliphatic carbonyl oxygen compounds. The conditions under which such derivative formation must take place necessitates the choice of a reagent for this purpose having the following characteristics:

(a) It must be soluble in the vegetable oil itself so that the oil may be used without appreciable dilution.

(b) The reaction between the reagent and the oxy-compounds which are to be removed from the oil, must be capable of going to completion so as to remove small traces of such compounds. The reaction should also be sensitive enough to take place at room temperature, thus avoiding the necessity of heating the oil to effect a reaction.

(c) The derivative so formed should have a low solubility in organic solvents. This is very desirable so that it may be washed free of oil without serious loss. The ideal derivative would be soluble in inorganic solvents only.

(d) The derivative should be able to undergo a complete decomposition to yield the original compound abstracted from the oil in quantitative amounts.

(e) The reagent should be applicable to extractions made from the oil by some organic solvent. This would aid materially in the separation of small amounts of compounds having differing solubilities.

It is conceded by everyone that a reagent which would fulfill each and all of the above requirements would be exceedingly difficult to find. It is highly improbable that such a convenient compound exists. However, the results of the above mentioned pioneering work show a very satisfactory reagent for the formation of pure derivatives of such oxy-compounds is found in the phenylhydrazine base. It is the most suitable of any of the numerous reagents tried. The type reaction between phenylhydrazine and the ozygen atoms of aldehydes and ketones has been well established by the monumental work of Emil Fischer on the sugars, proteins and uric acid. It is true that other reactions have been found to take place with phenylhydrazine and certain oxy-compounds, but this cannot be discussed in this short introductory paper.

Phenylhydrazine base fulfills the above desirable characteristics as follows: It is sufficiently soluble in vegetable oils to permit addition of an excess of the stoichiometric requirements. The reaction is conducted in the oil, practically undiluted. The reagent is added with an equal volume of glacial acetic acid, causing only a slight dilution of the oil. Solid derivatives are formed which may be filtered from the oil. This precipitation takes place at room temperature and requires about three days for completion. The hydrazones so produced are only slightly soluble in cold alcohol, ethyl ether and petroleum ether. They may be recrystallized from alcohol or glacial acetic acid yielding beautiful, pure compounds. The analysis and identification of these new compounds and reactions postulated for their formation in rancid vegetable oils will be the subject of another paper from the Mayonnaise Fellowship. This identification has been found to be difficult since the compounds involved do not appear to have been heretofore prepared. Due to the other investigations being conducted simultaneously, this report is now delayed.

This brings us to that part of this work in which all of us are most interested, namely, the possibility of using the above method of removing the offending compounds from a rancid oil *quantitatively*. In other words, if a method of analysis could be found whereby the exact amount of the offending compounds produced by the rancidification of an oil could be determined by forming removable compounds or derivatives, the rate of aging of a vegetable oil

[2]Scala, Gaz. Chim. Ital. *38*, 307-327, (1908).
[3]Nicolet, Ind. Eng. Chem. *8*, 416-417, (1916).

could be accurately followed. We have found from our preliminary experiments with the phenylhydrazones as derivatives that just this can be done.

Briefly, the method is based on the familiar principles, used in analytical chemistry, of adding an excess, in a known amount, of the reagent. The reaction desired is allowed to go to completion and the excess, unreacted reagent is then determined. The amount having entered into the reaction is then found by difference. In this case, the amount of phenylhydrazones formed stands in a simple stoichiometric relation to the oxy-compounds present. The actual determination of the excess phenylhydrazine used is made by the method first developed by Strache[4] and later refined by Riegler.[5]

This is done by quantitatively decomposing the unused phenylhydrazine in an alkaline solution of the oil by adding a copper sulphate solution. The copper oxide formed reacts with the phenylhydrazine base yielding free nitrogen which may be caught in a eudiometer over caustic and measured in the usual way. The reaction is conducted under an atmosphere of carbon dioxide.

[4]Strache, Monat. *12*, 524, (1891).
[5]Riegler, Zeit. Anal. Ch. *40*, 94-5, (1901).

Briefly, the results so far show that the amounts of those compounds which are capable of forming derivatives with phenylhydrazine increases as the oil ages and becomes rancid. This is true whether the aging is natural or accelerated by means of ultra-violet irradiation. The values appear to be continuous and show no irregularities even after the development of a decided organoleptic rancidity.

The foregoing is a short resumé of the type of work now being attempted by the Mayonnaise Fellowship plant at the Bureau of Chemistry and Soils. It is hoped that this paper will serve not only as an introduction for this new work on behalf of the Mayonnaise Manufacturers' Association, but that it will be considered by those who are already in this field of investigation as a cooperative program attacking the problem from a new angle. In such a spirit of collaboration, the Mayonnaise Association wishes to assume its just share of the work involved in the effort to increase the acceptable life of vegetable oils and the products into which they enter.

Acknowledgement is hereby given the kind generosity of the Capitol City Products Company of Columbus, Ohio, for supplying the cottonseed and corn oils used in this investigation.

(A paper by Dr. Kilgore read at the Fall meeting, giving even later developments, will be published in an early issue.)

Chemical Analysis, Tool and Training

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A PERIOD of belt tightening and bread lines, of diminishing or vanishing pay checks and disappearing departments is, perhaps, the best time to evaluate things as they actually are and to determine what are the ultimate necessities in the various lines of human activity. I haven't had either the time or courage to attempt the great indoor sport of questionnairing my colleagues, but I'm willing to hazard a guess that for every analytical laboratory that has fallen under the ax of the budget choppers, there are at least three research laboratories that have been cut off root and branch. While we all agree that the research laboratory is necessary to the future health and growth of industry, we know that the analytical laboratory is absolutely essential to preseve its very life; the operation of the factory is impossible without the help of the analysts. Research may be sacrificed to fill the gaping maw of the hungry creditors, but unless the factory is to stop functioning, the analysts must continue to turn out their daily quota of necessary control analyses.